spectroscopy (FCS). FCS is an advanced microscopy technique in which fluctuations in the fluorescence of a dye or dye-labeled molecule is recorded as the particles freely diffuse through a small focal volume. In this case, we measured the fluorescence of dye-labeled (Nile Red) HDL in the presence of wild type and mutant EL. The data can be analyzed mathematically using the cross-correlation function, from which the diffusion coefficient of the molecule is obtained. The lipase activity of EL changes HDL size, in turn affecting the diffusion coefficient, and can be calculated using the Stokes-Einstein relation. Our preliminary results suggest the hydrolysis of HDL occurs rapidly and proportionately to the concentration of EL. Several mutations in EL have been identified in human population studies. Our future goal is to compare the rate of hydrolysis between wild type and mutant EL and with normal and oxidized HDL. In addition, fluorescence measurements were used to investigate the composition of HDL. The fluorescence spectrum of Nile Red is dependent upon the local lipid environment. By monitoring the change in fluorescence emission as a function of EL metabolism, we investigate the content of HDL while being remodeled by EL. These fluorescence techniques allow us to answer some of the key questions regarding the HDL lipid collection and distribution function.

**3903-Pos**

**In Vivo Imaging of Single-Molecule Translocation through Nuclear Pore Complexes by Pair Correlation Functions**

**Francesco Cardarelli**, Enrico Gratton.
University of California, Irvine, CA, USA.

Nuclear pore complexes (NPCs) mediate bidirectional transport of proteins, RNAs, and ribonucleoproteins across the double-membrane nuclear envelope. We recently introduced a method based on pair correlation functions (pCF) which measure the time the same molecule takes to migrate from one location to another within the cell (1). The spatial and temporal correlation among two arbitrary points in the cell can provide a map of molecular transport, and also highlight the presence of barriers to diffusion with very high time resolution (in the microsecond scale) and spatial resolution (limited by diffraction).

Here we report the use of this method to directly monitor a model protein substrate undergoing transport through NPCs in living cells, a biological problem in which SPT has given results that cannot be confirmed by traditional FCS measurements because of the lack of spatial resolution. Our substrate is composed by a GFP linked to a functional nuclear localization sequence (NLS) and transfected into living CHO-K1 cells: the recombinant NLS-GFP protein can bind to molecular carriers mediating cytoplasm-to-nucleus active import as well as shuttle across the NPC by passive diffusion (its molecular weight is below the cut-off size limit of the NPC).

We show that obstacles to molecular flow can be detected and that the pCF algorithm can recognize the heterogeneity of NLS-GFP intracompartment diffusion as well as the presence of barriers to its transport between compartments (i.e. the NPCs of the nuclear envelope).

(1) Digman, M.A., and Gratton, E. Imaging Barriers to Diffusion by Pair Correlation Functions . Biophys. J. 97, 665-673 (2009).

Work supported in part by NIH-P41 P41-RRO3155 and P50-GM076516.

**3904-Pos**

**Fluorescence Correlation Spectroscopy for Clinical Testing in Von Willebrand Disease**

**Richard Torres**, Michael Levene.
Yale University, New Haven, CT, USA.

The von Willebrand factor (vWF) protein is an essential component of normal coagulation that is present in human plasma as a distribution of multimers composed of 2 to 40 or more monomers. Defects in the synthesis and metabolism of vWF represent the most common inherited abnormalities of coagulation and can be categorized as type 1 for quantitative deficiencies and type 2 for qualitative deficiencies. Current clinical methods for diagnosis and classification of von Willebrand disease suffer from significant limitations relating to the vast number of mutations that can occur, the fact that multimer size is a critical determinant of functional capacity, and the poor reproducibility of available activity assays. We have successfully employed the use of fluorescence correlation spectroscopy (FCS) to address the drawbacks of presently available vWF analysis methods. Autocorrelation curves from fluorescently tagged anti-vWF antibody incubated with plasma from normal donors and controls differ significantly from those obtained with plasma from patients with von Willebrand disease. Furthermore, it was possible to separate type 2 vWD patients from type 1 vWD patients on the basis of the shape and average diffusion time of the FCS curves. Cluster analysis yielded the expected separation of groups based on differences in the amount of antibody bound to antigen and the average diffusion time of bound antibody. Further analysis using a maximum entropy method FCS fitting program (MEMFCS) suggests further subclassification is possible with fluctuation analysis. The results indicate FCS is a practical tool for clinical evaluation of coagulopathic patients suspected of having von Willebrand disease. This research presents one of the first implementations of FCS in analysis of clinical samples.

**3905-Pos**

**Dynamic Imaging and Fluctuation Spectroscopy on Single Microvilli in Opossum Kidney Cells by the Modulation Tracking Method**

**Luca Lanzano**[1], Peter Fwu[1], Hector Giral[2], Moshe Levi[2], Enrico Gratton[1].
[1]Univ California, Irvine, CA, USA, [2]University of Colorado, Denver, CO, USA.

Regulation of renal tubular inorganic phosphate (Pi) transport occurs via the proximal tubular apical brush border membrane (BBM) sodium gradient-dependent Pi (NaPi) cotransport proteins. Distinct families of NaPi cotransporters show differential regulation under dietary and hormonal stimuli, but the way this is accomplished, for instance through localization in distinct BBM micro- or nano-domains and/or preferential interaction with different PDZ proteins, is not yet understood.

Crucial information could come from the application of single molecule fluctuation correlation spectroscopies on the BBM of living cultured Opossum Kidney (OK) cells expressing NaPi co-transporters with different GFP constructs. The BBM is composed of many microvilli, several micron long structures with a diameter of about 100nm. The microvilli show a relatively fast motion (in the seconds time scale) that makes the use of fluctuation spectroscopy difficult.

None of the current nano-resolution optical methods seems capable of measuring the clustering dynamics of proteins on the surface of rapidly moving microvilli. We developed an optical imaging technique called Modulation Tracking (MT) in which we track the center of mass of the microvillus at an arbitrary point along its length while the laser spot rapidly oscillates perpendicularly to the surface and the changes in the modulation are used to measure the distance of the spot from the fluorescent surface with nanometer resolution. High resolution images of the microvilli can be obtained scanning slowly along the microvillus axis. Since the moving microvillus is always at the center of the orbit, fluorescence image correlation techniques can be applied making the MT a truly dynamic nano-imaging technique.

Work supported in part by NIH RO1 DK066029-01A2 (ML, EG), NIH-P41 P41-RRO3155 (EG, PF and LL) and P50-GM076516 (EG, PF and LL).

**3906-Pos**

**Regulation of CFTR on the Plasma Membrane**

**Asmahan Abu-Arish**, Ian R. Bates, Elvis Pandzic, Angela Ho,
John W. Hanrahan, Paul W. Wiseman.
McGill University, Montreal, QC, Canada.

The cystic fibrosis transmembrane conductance regulator (CFTR) is an anion channel which is tightly regulated by phosphorylation and interactions with a macromolecular complex that mediates spatially localized signaling mechanisms. The complex may include scaffolds such as NHERF1, the adaptors ezrin and Receptor for Activated C Kinase (RACK1), and enzymes such as adenylyl cyclase, kinases, phosphatases, and phosphodiesterases. NHERF1 anchors CFTR to the actin cytoskeleton whereas RACK1 mediates its association with protein kinase C (PKC), however the relationships between these proteins remain poorly understood. We have studied the dynamics of fluorescent fusion proteins containing CFTR, NHERF1 and RACK1 using quantitative fluorescence fluctuation imaging techniques. Lateral diffusion coefficients and immobility fractions at the plasma membrane were calculated from time-series of confocal images using temporal image correlation spectroscopy (TICS). We also developed a novel cross-correlation TICS (CC-TICS) analysis for studying the dynamics of interacting protein species and their binding ratios, so that the assembly and disassembly of the CFTR regulatory complex could be studied quantitatively. Initial results indicate that the lateral mobilities of RACK1 ($D=1.5\pm0.6\ \xi10^{-3}\ \mu m^2/s$) and NHERF1 ($D=2.6\pm1\ \xi10^{-4}\ \mu m^2/s$) are both significantly reduced ($D=8.6\pm2.5\ \xi10^{-4}$ and $1.8\pm0.8\ \xi10^{-4}\ \mu m^2/s$, respectively) when co-expressed with CFTR and are further reduced upon activation of PKC ($D=6.6\pm2.2\ \xi10^{-4}$ and $1\pm0.4\ \xi10^{-4}\ \mu m^2/s$, respectively). The fractions of immobility significantly increased whenever the diffusion coefficient decreased. The results suggest two distinct phases during CFTR complex formation; initial tethering under basal conditions followed by aggregation into complexes during PKC stimulation. These preliminary results provide new insight into protein-protein interactions that regulate CFTR, information that is essential for understanding anion transport in cystic fibrosis and secretory diarrhea.

**3907-Pos**

**Predicting Protein Co-Expression Fractions in Living Cells**

**Elizabeth M. Smith**, Yan Chen, Joachim Mueller.
University of Minnesota, Minneapolis, MN, USA.

Fluorescence fluctuation spectroscopy utilizes the fluctuation in a fluorescent signal to determine molecular brightness, concentration, and diffusion properties of fluorescent particles passing through an optical volume. Brightness analysis is